



It's about time

TOPO[®] PCR cloning technology



TOPO[®] PCR cloning technology

- Efficient—up to 95% of clones contain desired insert
- Easy—3-step procedure
- Fast—5-minute, room temperature reaction
- Proven—over 4,000 citations

More free time and better cloning efficiency in just three simple steps

TOPO[®] cloning technology removes time-intensive and unreliable steps from your cloning workflow, allowing you to perform benchtop cloning reactions in just 5 minutes. With up to 95% recovery of your desired clone, you always have the clone you need for downstream experiments. With more than 10 years of established performance and over 4,000 scientific citations, TOPO[®] cloning is the method of choice for researchers around the world. Whether you're performing general subcloning, sequencing, or *in vitro* transcription; expression in *E. coli* or mammalian cells; or using the Gateway[®] system, there's a TOPO[®] cloning solution for you. Fast, reliable, and direct, TOPO[®] cloning gets the right clone in your hands sooner, freeing up your time to answer more important questions.

Table of contents

TOPO® cloning technology 4

Select your TOPO® kit by application 6

TOPO® PCR cloning kits for:

- Subcloning. 8
- Sequencing 9
- Blunt-fragment cloning 10
- Long-fragment cloning 11

Directional TOPO® cloning 12

TOPO® cloning for *E. coli* expression 13

TOPO® cloning for mammalian expression. 14

High-throughput TOPO® cloning. 15

Custom TOPO® services. 15

TOPO® cloning into the Gateway® system 16

Ordering information 18



The technology behind TOPO® cloning

The key to TOPO® cloning is the enzyme DNA topoisomerase I, which functions both as a restriction enzyme and as a ligase. Its biological role is to cleave and rejoin DNA during replication. *Vaccinia* virus topoisomerase I specifically recognizes the pentameric sequence 5'-(C/T)CCTT-3' and forms a covalent bond with the phosphate group attached to the 3' thymidine. It cleaves one DNA strand, enabling the DNA to unwind. The enzyme then reli-

gates the ends of the cleaved strand and releases itself from the DNA. To harness the religating activity of topoisomerase, TOPO® vectors are provided linearized with topoisomerase I covalently bound to each 3' phosphate. This enables the vectors to readily ligate DNA sequences with compatible ends (Figures 1, 2, and 3). The ligation is complete in only 5 minutes at room temperature.

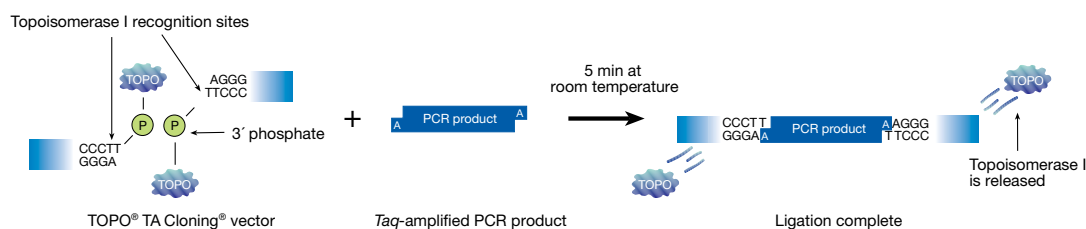


Figure 1—TOPO® TA Cloning® of Taq-amplified DNA.

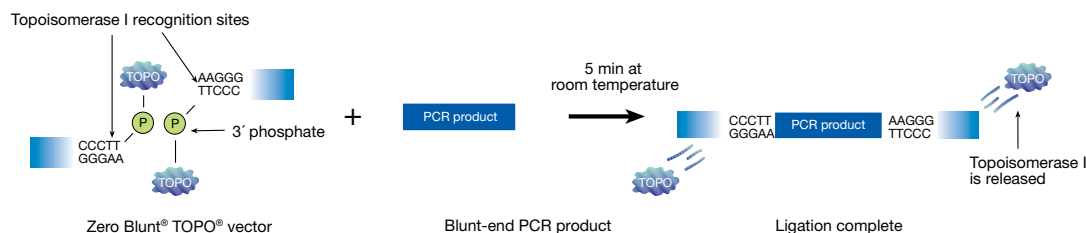


Figure 2—Zero Blunt® TOPO® cloning of blunt-end DNA.

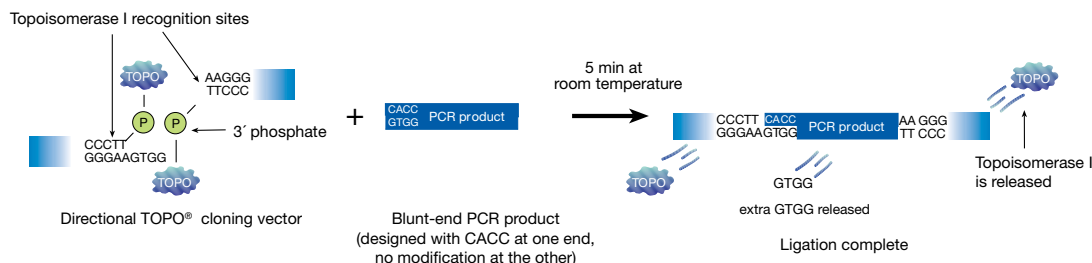


Figure 3—Directional TOPO® cloning of blunt-end DNA.

Three simple steps save time

TOPO® PCR cloning requires just three easy steps. Simply combine your PCR product and a TOPO® cloning vector in the provided solution, wait 5 minutes, then transform *E. coli* (Figure 4). With TOPO® cloning, the additional time, steps, and reagents required

for ligase-mediated cloning are eliminated. Table 1 provides conservative estimates of the time saved using TOPO® cloning versus other methods.

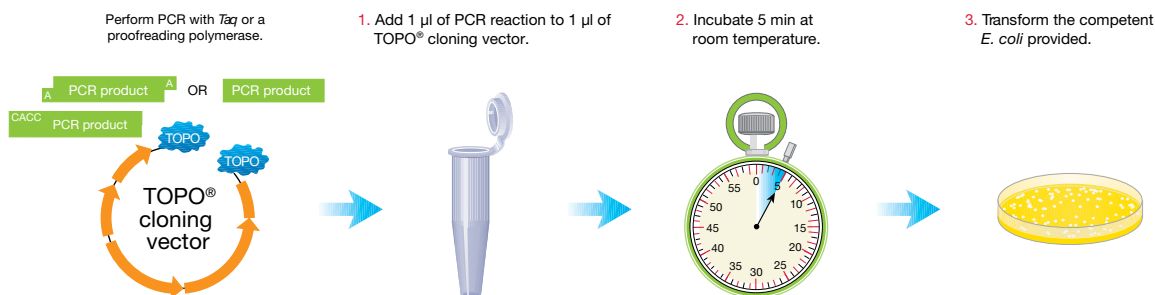


Figure 4—The TOPO® cloning protocol.

Table 1—TOPO® TA Cloning® Kits deliver the highest efficiency in the shortest time.

Steps	TOPO® TA Cloning® Kits	TA/UA cloning	Restriction enzyme or cut-back cloning
Use of existing primers?	Yes	Yes	No
Vector is ready for cloning?	Yes	Yes	No
Ligation reagents included?	Yes	Yes	No
Prepare or purchase competent cells separately?	No, included	Purchase: 0 hr Prepare: up to 6 hr	Purchase: 0 hr Prepare: up to 6 hr
Ligation time	5 min	1 hr	2–23 hr
Efficiency	Up to 95%	60–80%	~60%
Time required for cloning	5 min	1–12 hr	2–23 hr



Select your TOPO® kit by application

Whether you're PCR cloning with *Taq* DNA polymerase or a proofreading enzyme, there is a TOPO® cloning kit available to

take you quickly and efficiently to your downstream application (Table 2). For a complete list of products, please visit our website at www.invitrogen.com/topo.

Table 2—Select the right TOPO® cloning kit.

Application of cloned PCR product	Product	Vector backbone	TA, blunt, or directional	Key advantages	Competent <i>E. coli</i>	Cat. no.
General subcloning	TOPO® TA Cloning® Kit	pCR®2.1 TOPO®	TA	<ul style="list-style-type: none">• Get your clone—95% efficiency• Selection of competent cells for fast growth, electroporation, or routine cloning	One Shot® Mach1™-T1 ^R	K4510-20
					One Shot® TOP10	K4500-01
					One Shot® TOP10F'	K4550-01
					One Shot® TOP10 Electrocomp™	K4560-01
	Zero Blunt® TOPO® PCR Cloning Kit	pCR®-Blunt II TOPO®	Blunt	<ul style="list-style-type: none">• Unique technology to minimize background• Get your clone—95% efficiency• Multiple primer sites (T7, T3, M13F, M13R) make sequence analysis or PCR easy and convenient• Selection of competent cells for fast growth, electroporation, or routine cloning	One Shot® Mach1™-T1 ^R	K2830-20
					One Shot® TOP10	K2800-20
Long PCR fragments	TOPO® XL PCR Cloning Kit	pCR®-XL-TOPO®	TA	<ul style="list-style-type: none">• High-efficiency cloning for fragments 3–10 kb• Unique technology to minimize background	One Shot® Mach1™-T1 ^R	K7030-20
					One Shot® TOP10	K4750-10
					One Shot® TOP10 Electrocomp™	K4700-10
<i>In vitro</i> transcription	TOPO® TA Cloning® Kit Dual Promoter	pCR®II TOPO®	TA	<ul style="list-style-type: none">• Get your clone—95% efficiency• Dual T7 and SP6 priming sites for <i>in vitro</i> transcription	One Shot® Mach1™-T1 ^R	K4610-20
					One Shot® TOP10F'	K4600-01
					One Shot® TOP10 Electrocomp™	K4660-01
Sequencing	TOPO® TA Cloning® Kit for Sequencing	pCR®4 TOPO®	TA	<ul style="list-style-type: none">• Get your clone—95% efficiency• Minimal MCS (multiple cloning site) so you sequence more insert, less vector• Multiple primer sites (T7, T3, M13F, M13R) for sequence analysis	One Shot® Mach1™-T1 ^R	K4530-20
					One Shot® TOP10	K4575-01
					One Shot® TOP10 Electrocomp™	K4580-01
	Zero Blunt® TOPO® PCR Cloning Kit for Sequencing	pCR®4Blunt TOPO®	Blunt	<ul style="list-style-type: none">• Get your clone—95% efficiency• Minimal MCS so you sequence more insert, less vector• Unique technology to minimize background	One Shot® Mach1™-T1 ^R	K2835-20
					One Shot® TOP10	K2875-20
					One Shot® TOP10 Electrocomp™	K2880-20

Application of cloned PCR product	Product	Vector Backbone	TA, blunt, or directional	Key advantages	Competent <i>E. coli</i>	Cat. no.
Expression in <i>E. coli</i>	Champion™ pET100 Series Directional TOPO® Expression Kits	pET D-TOPO®	Directional	<ul style="list-style-type: none">• Up to 90% of clones in the correct orientation• High-level protein expression using <i>E. coli</i>• Inducible expression using IPTG• 6xHis tag for convenient purification and detection• EK cleavage sequence	BL21 Star™(DE3) One Shot®	K100-01
						K101-01
						K102-01
						K151-01
Expression in mammalian cells	pcDNA™3.3-TOPO® TA Cloning® Kit	pcDNA™	TA	<ul style="list-style-type: none">• 3–5x higher expression compared to conventional CMV vectors• Ideal for expression of native protein, no extra amino acids	One Shot® TOP10	K8300-01
	pcDNA™3.2/V5/GW/D-TOPO® Cloning Kit	pcDNA™	Directional	<ul style="list-style-type: none">• >90% of clones in correct orientation• CMV promoter delivers high-level expression in mammalian cells• C-terminal V5 and 6xHis tags for convenient detection and purification• Neomycin selection	One Shot® TOP10	K2440-20
	ViraPower™ HiPerform™ Lentiviral Expression Kits	pLenti7.3	TA	<ul style="list-style-type: none">• >4x higher expression compared to the original system• Efficient TOPO® cloning• Includes Fast Titering module	One Shot® Stbl3™	K5320-00
		pLenti6.3				K5310-00
	Entry into Gateway® systems	pCR®8/GW/TOPO® TA Cloning® Kit	pCR®8	TA	<ul style="list-style-type: none">• Maximum convenience—including plasmid miniprep• Fast-growing Mach1™ <i>E. coli</i> shortens cloning• Fast-growing <i>E. coli</i> shortens cloning time	Mach1™-T1 ^R
						K2520-20
					One Shot® TOP10	K2500-20
pENTR™/TEV/D-TOPO® Cloning Kit		pENTR™ D-TOPO®	Directional	<ul style="list-style-type: none">• Fast-growing <i>E. coli</i> shortens cloning time• 5´ TEV sequence for N-terminal tag removal creates native proteins	Mach1™-T1 ^R	K2535-20
pENTR™/TEV/D-TOPO® Cloning Kit				<ul style="list-style-type: none">• 5´ TEV sequence for N-terminal tag removal creates native proteins		K2525-20
pENTR™/SD/D-TOPO® Cloning Kit				<ul style="list-style-type: none">• Includes Shine-Dalgarno sequence for <i>E. coli</i> expression—ready entry clone	One Shot® TOP10	K2420-20
pENTR™/D-TOPO® Cloning Kit						K2400-20



Subcloning

For fast, efficient subcloning in a variety of applications, the only choice is TOPO® cloning technology, which eliminates time-consuming and tedious restriction site cut-back cloning. TOPO® cloning is hands down the most reliable method, taking only 5 minutes and yielding up to 95% recombinants. Convenient features of the pCR®-TOPO® vectors (Figure 5) include:

- *Eco*R I sites flanking the PCR product insertion site for easy removal of inserts
- Kanamycin and ampicillin resistance genes for your choice of selection in *E. coli*
- Easy blue/white screening of recombinant colonies
- T7 and M13 (pCR®2.1-TOPO®) or T7 and SP6 (pCR®II-TOPO®) promoter/priming sites for *in vitro* transcription
- M13 forward (–20) and reverse priming sites for sequencing or PCR screening

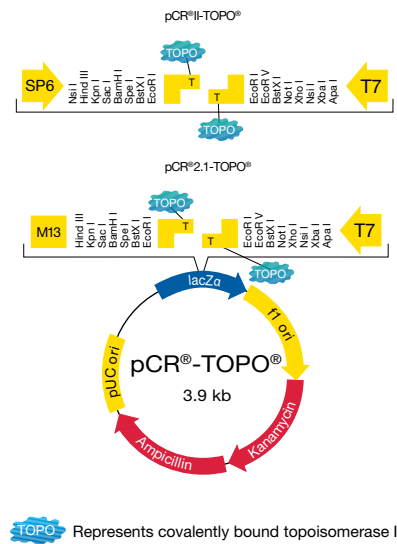


Figure 5—pCR®-TOPO® vectors for subcloning.

Save an entire day

With Mach1™-T1^R *E. coli*, the fastest-growing cloning strain, you can achieve sufficient growth for minipreps from an overnight colony in just four hours—and save an entire day over cloning with most other strains (Table 3). Mach1™-T1^R cells offer several genotypic advantages, including *recA* for reduced occurrence of unwanted recombination, *tonA1* for resistance to T1 and T5 phages, *endA1* for cleaner DNA preparations, and *lacZΔM15* for blue/white screening of colonies. We offer a suite of TOPO® cloning kits with Mach1™-T1^R cells for subcloning, sequencing, long-fragment PCR cloning, and entry into the Gateway® system.

Table 3—TOPO® cloning kits with Mach1™-T1^R competent cells save time.

	TOPO® cloning kits with Mach1™-T1 ^R cells	TOPO® cloning kits with TOP10F', DH5α™ cells	Restriction enzyme or cut-back cloning
Day 1	Cloning and ligation Transformation	Cloning and ligation Transformation	Cloning and ligation
Day 2	Miniprep DNA Purify plasmid DNA	Miniprep DNA	Transformation
Day 3		Purify Plasmid DNA	Miniprep DNA
Day 4			Purify plasmid DNA

Sequencing

The TOPO® cloning kits for sequencing allow fast cloning and streamlined sequencing of PCR products. These kits contain TOPO® cloning vectors with a minimized multiple cloning site that positions the T7 and T3 priming sites only 33 bp away from the PCR product insertion site (Figure 6). This means you'll sequence more of your insert and less of the vector.

Choice of vector

The pCR®4-TOPO® vector supplied in the TOPO® TA Cloning® Kit for Sequencing has 3'-T overhangs for cloning *Taq*-amplified PCR products. The pCR®4Blunt-TOPO® vector supplied in the Zero Blunt® TOPO® PCR Cloning Kit for Sequencing has blunt ends for cloning PCR products amplified with proofreading polymerases.

Convenient features of these sequencing vectors include:

- T7 and T3 promoter/priming sites for sequencing and *in vitro* transcription/translation
- M13 forward (–20) and reverse priming sites for sequencing or PCR screening
- The *ccdB* gene to eliminate background and improve results
- *EcoR* I sites flanking the PCR product insertion site for easy removal of inserts
- Unique *Sse8387* I site to produce nested deletions for sequencing internal regions of your insert
- Kanamycin and ampicillin resistance genes for your choice of selection in *E. coli*

Maximize transformation

Both kits are offered with Mach1™-T1^R competent cells for the fastest cloning. The Mach1™-T1^R *E. coli* strain is the fastest-growing cloning strain, allowing you to achieve sufficient growth for mini-preps from an overnight colony in just four hours, which saves an entire day over most other cloning strains. We also offer both TOPO® TA Cloning® Kits for sequencing with our highly popular TOP10F' or MAX Efficiency® DH5α™-T1 Phage-Resistant competent cells.

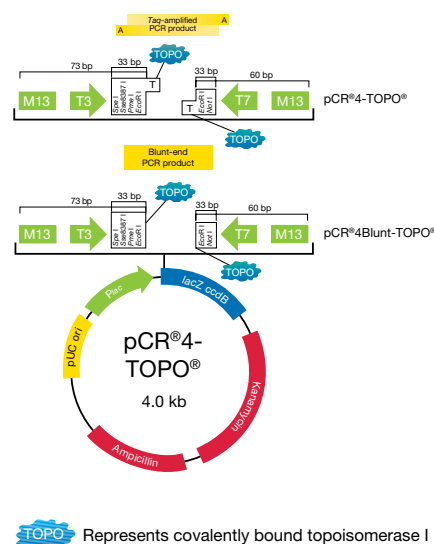


Figure 6—pCR®4-TOPO® cloning vectors for sequencing.

Blunt-fragment cloning

The Zero Blunt® TOPO® PCR Cloning Kit combines the unique Zero Background™ technology (see box below) with the pCR®-Blunt II-TOPO® vector (Figure 7) to allow easy, high-efficiency cloning of blunt-end PCR products. Convenient features of this vector include:

- *ccdB* gene to eliminate background of self-ligated vectors
- *EcoR* I sites flanking the PCR product insertion site for easy removal of inserts
- Kanamycin and Zeocin™ resistance genes for your choice of selection in *E. coli*
- T7 and SP6 promoter/priming sites for *in vitro* RNA transcription and sequencing
- M13 forward (–20) and reverse priming sites for sequencing or PCR screening
- Selection of competent cells for high speed, electroporation, or routine cloning

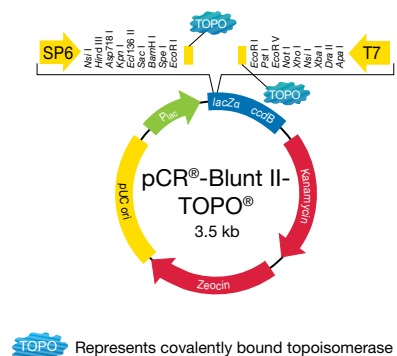


Figure 7—pCR®-Blunt II-TOPO® vector.

Eliminate high background

Because of high background, cloning blunt-end and long PCR products can be difficult and often yields a low percentage of recombinants. Invitrogen's unique Zero Background™ technology uses the lethal *ccdB* (control of cell death) gene to enable high-efficiency cloning, yielding nearly 100% recombinants. The *ccdB* protein poisons bacterial DNA gyrase, causing degradation of the host chromosome and cell death.^{1,2} When an insert is ligated into the vector, the *ccdB* gene is disrupted, enabling only recombinant colonies to grow (Figure 8).

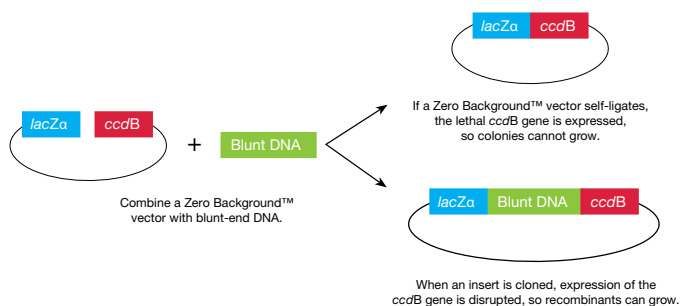


Figure 8—Zero Background™ technology enables recovery of only recombinant clones.

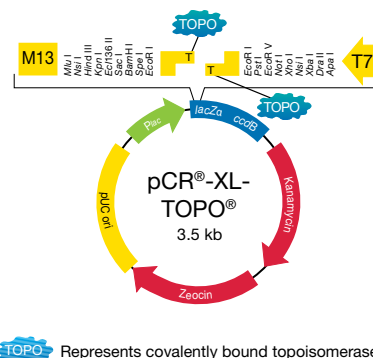
Long-fragment cloning

The TOPO® XL PCR Cloning Kit combines TOPO® cloning, Zero Background™ technology, and a unique gel purification step to enhance cloning of PCR products from 3 to 10 kb. Convenient features of the pCR®-XL-TOPO® vector (Figure 9) include:

- *ccdB* gene to eliminate background
- Kanamycin and Zeocin™ resistance genes for your choice of selection in *E. coli*
- T7 promoter/priming site for *in vitro* RNA transcription and sequencing
- M13 forward (–20) and reverse priming sites for sequencing or PCR screening
- Selection of competent cells for high speed, electroporation, or routine cloning

Unique gel purification step improves results

Long PCR often yields multiple products, making gel purification necessary prior to cloning. However, gel purification usually involves exposure to ethidium bromide and UV light, which can nick and damage DNA.³ To protect against nicking, the TOPO® XL PCR Cloning Kit uses crystal violet to enable visualization of DNA bands in an agarose gel in ambient light. This eliminates the need for ethidium bromide and UV light exposure, ensuring safe gel purification. Crystal violet staining results in significantly more colonies and a greater percentage of recombinants than using ethidium bromide and UV light (Table 4).



TOPO® Represents covalently bound topoisomerase I

Figure 9—pCR®-XL-TOPO® vector.

Table 4—Crystal violet enhances TOPO® cloning of large fragments. A 7 kb ampicillin resistance gene sequence was PCR amplified, and PCR products were loaded onto gels stained with crystal violet or ethidium bromide. PCR products were gel purified and cloned into the pCR®-XL-TOPO® vector. The number of recombinants was determined by plating 125 µl of each transformation on LB plates containing either kanamycin or kanamycin and ampicillin.

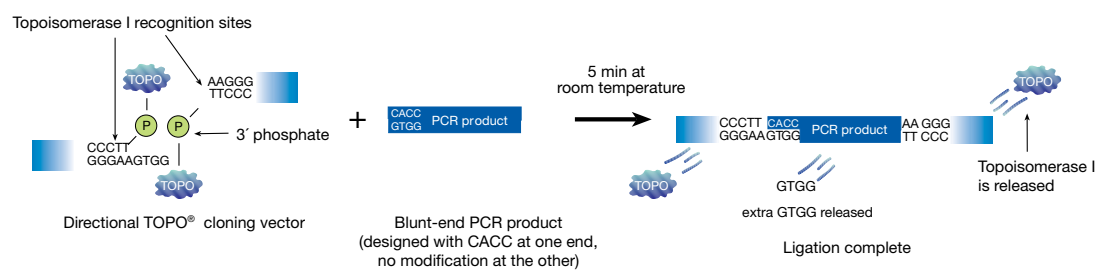
	Crystal violet	Ethidium bromide
Total colonies (Kan ^R)	275	15
Colonies w/insert (Kan ^R + Amp ^R)	258	9
Percent recombinants	94%	60%



Directional TOPO® cloning

Directional TOPO® cloning enables directional cloning of blunt-ended PCR products directly into an expression vector using a 5-minute ligation reaction, thereby eliminating subcloning steps and saving you time. Directional TOPO® cloning vectors have a single-stranded GTGG overhang at one end and a blunt end at the other. The four-nucleotide overhang invades the double-stranded DNA of the PCR product and anneals to the CACC sequence that you place in your primer. Topoisomerase I then ligates the PCR product in the correct orientation for expression. With Directional TOPO® Cloning Expression Kits, you will:

- Save time—TOPO® cloning of your PCR product takes just five minutes
- Obtain efficient cloning results—recombinant clones (>90%) will be in the correct orientation for expression (Figure 10)
- Achieve high-level expression—vectors carry powerful promoters for expression in *E. coli* or mammalian cells



Clone	No. in correct orientation	No. in reverse orientation	% Correct
D32129 (1,171 bp)	18	2	90%
AF016582 (1,504 bp)	20	0	100%
AF020833 (1,036 bp)	19	1	95%

Figure 10—Schematic of directional TOPO® cloning, and results of directional cloning of human open reading frames into pcDNA™3.1D/V5-His-TOPO® vector.

TOPO® cloning for *E. coli* expression

Champion™ pET Directional TOPO® vectors are powerful *E. coli* expression vectors that use the highly efficient T7 RNA polymerase to achieve strong transcription levels and high protein yields. T7 RNA polymerase is expressed by host *E. coli* under the control of the IPTG-inducible *lacUV5* promoter. This allows you to regulate transcription with IPTG. The additional *lacO* element found in the

T7 *lac* promoter used in the pET vectors further reduces the basal expression levels while enabling strong transcriptional activity upon induction with IPTG. Reported yields of recombinant proteins from the pET vectors are typically in the range of tens to hundreds of milligrams per liter of culture (Figure 11).

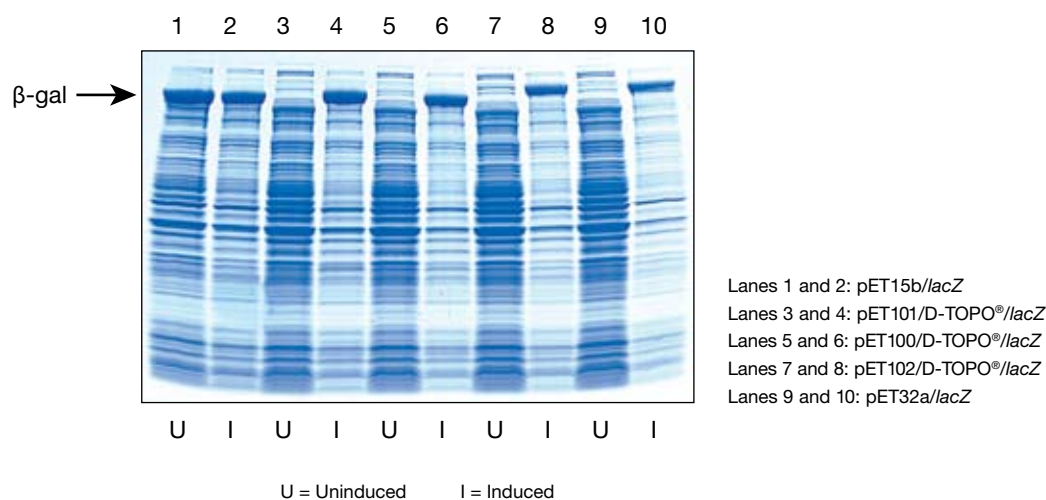


Figure 11—Strong induced expression with pET Directional TOPO® vectors. The *lacZ* gene was cloned directionally into pET100/D-TOPO®, pET101/D-TOPO®, and pET102/D-TOPO® vectors and cloned using the restriction digest method into pET15b and pET32a vectors. Constructs were transformed into BL21 Star™(DE3) *E. coli*. A single colony from each transformation was used to inoculate 1 ml of LB medium supplemented with 100 µg/ml ampicillin. Cultures were induced with 1 mM IPTG at OD₆₀₀ = 0.5. At 2.5 hr postinduction, cultures were harvested by centrifugation. Pellets were resuspended in 300 µl sample buffer. Ten microliters of each sample was analyzed on a 4–20% Novex® Tris-glycine gel. Note: pET15b contains an N-terminal 6xHis tag, while pET32a contains an N-terminal thioredoxin fusion and a C-terminal 6xHis tag.

TOPO® cloning for mammalian expression

For constitutive mammalian expression, the pcDNA™ mammalian expression vector is one of the most popular expression vectors available today. The newest version is the pcDNA™3.3-TOPO® vector (Figure 12), which enables expression of exceptionally high levels of recombinant protein in adherent or suspension-adapted mammalian cells, and is ideal for use with Invitrogen's FreeStyle™ Expression Systems.

- Two- to five-fold higher protein yields compared to other expression vectors
- Fast, efficient, simple cloning using TOPO® TA technology
- Express native (or tagged) proteins without extraneous amino acids—ideal for antibody production and structural biology

ViraPower™ HiPerform™ Lentiviral Expression Systems

The ViraPower™ HiPerform™ Lentiviral Expression Systems provide stable gene expression and reproducible delivery to both dividing and nondividing cells. The lentiviral expression system enables:

- Greater than four-fold increase in protein expression
- Efficient gene delivery into cells that are virtually impossible to transfect
- Accurate and fast 2-day titering of functional lentivirus
- Selection of Gateway® or TOPO® TA cloning vectors

(Figure 13)

The ViraPower™ expression systems provide you with the high levels of stable gene expression necessary for valid results in virtually any cell line, especially in primary or difficult-to-transfect cells (Figure 14).

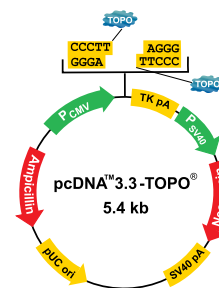


Figure 12—General features of the pcDNA™3.3-TOPO® mammalian expression vector.

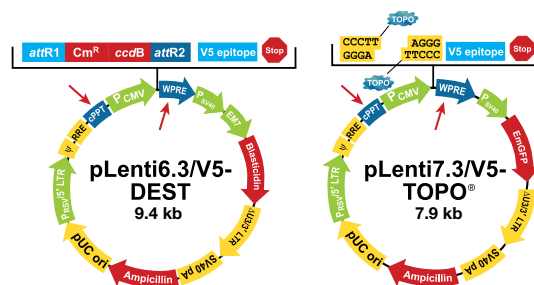


Figure 13—ViraPower™ HiPerform™ vectors. Maps of the pLenti6.3/V5-DEST Gateway® vector and the pLenti7.3/V5-TOPO® vector are shown. Both vectors are available in TOPO® TA and Gateway® formats. Red arrows indicate WPRE and cPPT elements.

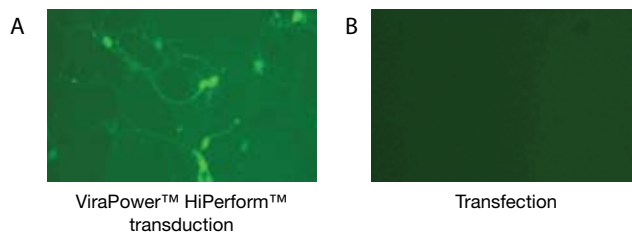


Figure 14—GFP expression in neuronal cells transduced with pLenti6.3/V5/GW/EmGFP packaged using a ViraPower™ HiPerform™ kit. Cells were transiently transfected with pLenti6.3/V5/GW/EmGFP or transduced with lentiviral particles prepared from the same vector. EmGFP expression was visualized 40 hours after transduction or transfection.

High-throughput TOPO® cloning

HTP TOPO® cloning kits couple TOPO® technology and a high-throughput format, enabling you to easily and simultaneously clone thousands of PCR products. With 480 reactions of TOPO® vector supplied in a single tube, you can quickly set up your TOPO® reactions in 96-well plates or strip-well formats, incubate for only 5 minutes, then transform competent *E. coli* using a multichannel pipette. With the speed and high efficiency of TOPO® cloning, you'll not only get your clones fast, you'll get them the first time, eliminating time wasted repeating unsuccessful reactions. Contact Technical Support to learn more.

Custom TOPO® services

The development of gene-based therapeutic and diagnostic products requires the rapid analysis of a vast number of gene sequences. When screening gene targets that are of commercial importance, being the first to identify, clone, express, and validate these genes is crucial. Invitrogen's Custom TOPO® Cloning Adaptation Service puts the power of TOPO® cloning into your vector. With your own vector adapted to TOPO® technology, you can:

- Save time—TOPO® cloning takes only 5 minutes and is so effective, you won't have to repeat experiments
- Maintain your current experimental strategy—adapting your own vector for TOPO® cloning doesn't change your downstream studies, but it will get you there faster

TOPO® cloning into the Gateway® system

We offer several TOPO® cloning kits that allow direct access to our Gateway® expression system. Our pCR®8/GW/TOPO® vector (Figure 15A) offers streamlined sequence analysis so you sequence more of your insert and less of the vector. With our patented *att* sites, the pCR®8 vector serves as a convenient entry vector to the Gateway® system, allowing you to easily access a variety of expression vectors without tedious subcloning steps, and saving you valuable research time.

The pENTR™ vector series (Figure 15B) provides Directional TOPO® cloning, capturing your insert in the correct orientation for protein expression. Additionally, our pENTR™/TEV/D-TOPO® vector has a tobacco etch virus (TEV) recognition site for efficient cleavage of any N-terminal tag and 5' *att* site after recombination with the destination vector. pcDNA™/V5/GW/D-TOPO® vectors (Figure 15C) are also available for high-level mammalian expression followed by possible analysis in other systems.

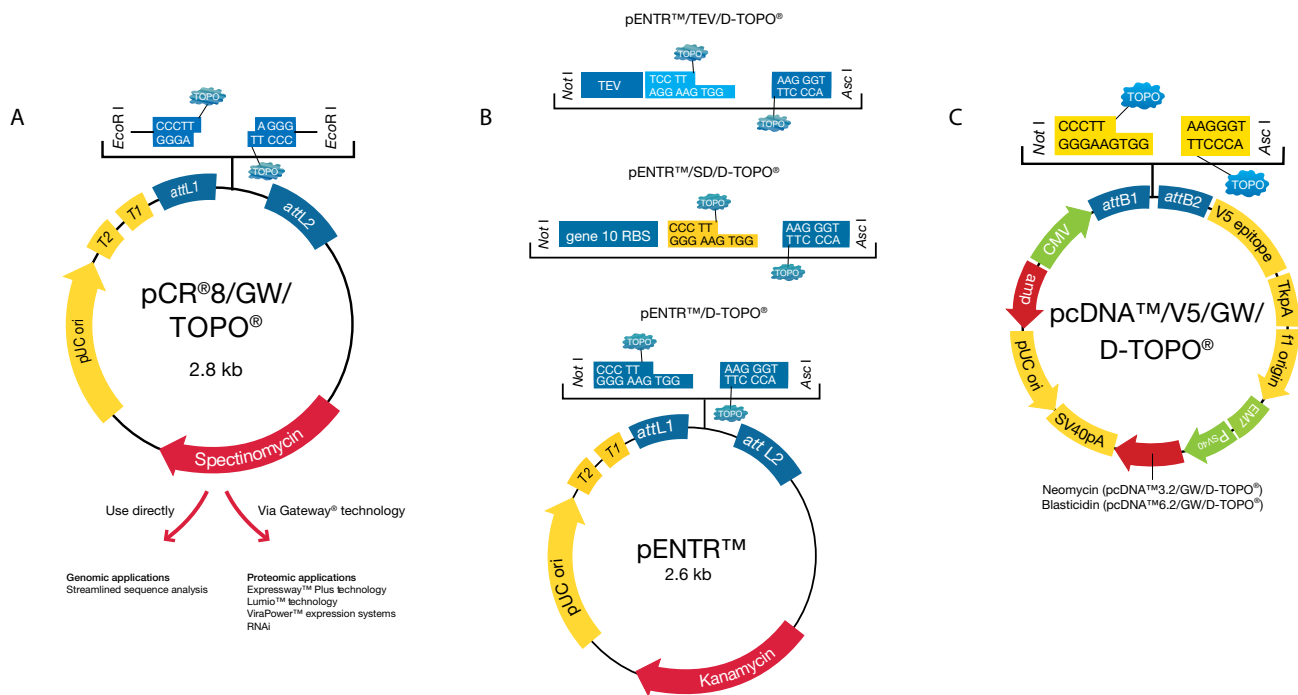


Figure 15—Multiple entry vectors for the Gateway® system. **A.** The pCR®8/GW/TOPO® entry vector allows TOPO® TA Cloning® for multiple downstream applications. **B.** Several pENTR™ vectors are available for directional TOPO® cloning and direct access to the multitude of Gateway® expression vectors. **C.** The pcDNA™/GW/D-TOPO® expression vector allows directional TOPO® cloning, mammalian expression, and access to the Gateway® system.

Rapid cloning for multiple applications

The Gateway® expression system is a powerful technology designed to simplify cloning and provide a rapid and highly efficient route to multiple expression and functional analysis options. There are many ways to clone a gene, but only Gateway® technology gives you ultimate access to multiple expression options (Figure 16). Rapidly transfer your gene and express it in as many systems as you choose. Take the first step towards accessing all of your expression needs by simply cloning your gene into a Gateway® entry vector. Whether you prefer TOPO®, PCR, or restriction cloning, we have a Gateway® entry vector for you. Visit www.invitrogen.com/gateway to learn more about our Gateway® technology.

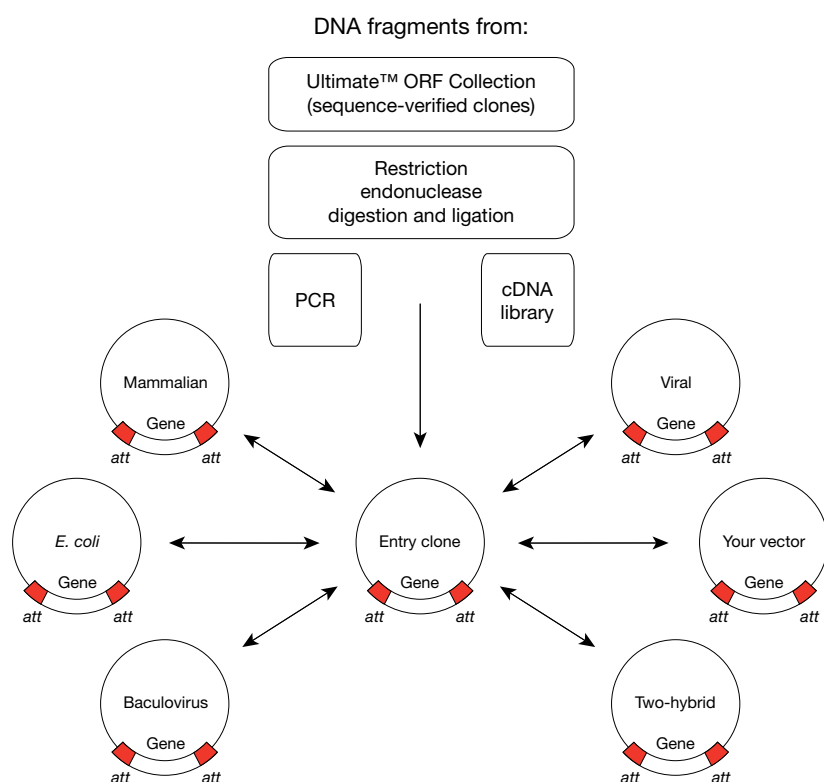


Figure 16—The flexibility of Gateway® technology. Gateway® technology is a powerful system designed to simplify cloning and provide a rapid and highly efficient route to multiple expression and functional analysis options.



Ordering information

Application of cloned PCR product	Product	With competent <i>E. coli</i> (+ plasmid miniprep)	Cat. no.	Number of rxns
General subcloning	TOPO® TA Cloning® Kit	One Shot® Mach1™-T1 ^R	K4510-20	20
		One Shot® Mach1™-T1 ^R + PureLink™ Quickprep Plasmid Miniprep Kit	K4510-22	20 rxns + preps
		One Shot® TOP10	K4500-01	20
		One Shot® TOP10	K4500-40	40
		One Shot® TOP10 + PureLink™ Quickprep Plasmid Miniprep Kit	K4500-02	20 rxns + preps
		One Shot® TOP10F'	K4550-01	20
		One Shot® TOP10F'	K4550-40	40
		One Shot® TOP10 Electrocomp™	K4560-01	20
		One Shot® TOP10 Electrocomp™	K4560-40	40
		One Shot® MAX Efficiency® DH5α™-T1 Phage-Resistant	K4520-01	20
		One Shot® MAX Efficiency® DH5α™-T1 Phage-Resistant	K4520-40	40
	Zero Blunt® TOPO® PCR Cloning Kit	One Shot® Mach1™-T1 ^R	K2830-20	20
		One Shot® TOP10	K2800-20	20
		One Shot® TOP10	K2800-40	40
		One Shot® TOP10 Electrocomp™	K2860-20	20
		One Shot® MAX Efficiency® DH5α™-T1 Phage-Resistant	K2820-20	20
Long PCR fragment cloning	TOPO® XL PCR Cloning Kit	One Shot® Mach1™-T1 ^R	K7030-20	20
		One Shot® TOP10	K4750-10	10
		One Shot® TOP10	K4750-20	20
		One Shot® TOP10 Electrocomp™	K4700-10	10
		One Shot® TOP10 Electrocomp™	K4700-20	20
<i>In vitro</i> transcription	TOPO® TA Cloning® Dual Promoter Kit	One Shot® Mach1™-T1 ^R	K4610-20	20
		One Shot® TOP10F'	K4600-01	20
		One Shot® TOP10F'	K4600-40	40
		One Shot® TOP10F'	K4650-01	20
		One Shot® TOP10F'	K4650-40	40
		One Shot® TOP10 Electrocomp™	K4660-01	20
		One Shot® TOP10 Electrocomp™	K4660-40	40
		One Shot® MAX Efficiency® DH5α™-T1 Phage-Resistant	K4620-01	20
		One Shot® MAX Efficiency® DH5α™-T1 Phage-Resistant	K4620-40	40
Sequencing	TOPO® TA Cloning® Kit for Sequencing	One Shot® Mach1™-T1 ^R	K4530-20	20
		One Shot® TOP10	K4575-J10	10
		One Shot® TOP10	K4575-01	20
		One Shot® TOP10	K4575-40	40
		One Shot® TOP10 + PureLink™ Quickprep Plasmid Miniprep Kit	K4575-02	20
		One Shot® TOP10 Electrocomp™	K4580-01	20
		One Shot® TOP10 Electrocomp™	K4580-40	40
		One Shot® MAX Efficiency® DH5α™-T1 Phage-Resistant	K4595-01	20
		One Shot® MAX Efficiency® DH5α™-T1 Phage-Resistant	K4595-40	40
	Zero Blunt® TOPO® PCR Cloning Kit for Sequencing	One Shot® Mach1™-T1 ^R	K2835-20	20
		One Shot® TOP10	K2875-J10	10
		One Shot® TOP10	K2875-20	20
		One Shot® TOP10	K2875-40	40
		One Shot® MAX Efficiency® DH5α™-T1 Phage-Resistant	K2895-20	20
		One Shot® TOP10 Electrocomp™	K2880-20	20
		One Shot® TOP10 Electrocomp™	K2880-40	40

Application of cloned PCR product	Product	With competent <i>E. coli</i> (+ plasmid miniprep)	Cat. no.	Number of rxns
Expression in <i>E. coli</i>	Champion™ pET100 Directional TOPO® Expression Kits	BL21 Star™(DE3) One Shot®	K100-01	20
	Champion™ pET101 Directional TOPO® Expression Kits	BL21 Star™(DE3) One Shot®	K101-01	20
	Champion™ pET102 Directional TOPO® Expression Kits	BL21 Star™(DE3) One Shot®	K102-01	20
	Champion™ pET151 Directional TOPO® Expression Kits	BL21 Star™(DE3) One Shot®	K151-01	20
	Champion™ pET200 Directional TOPO® Expression Kits	BL21 Star™(DE3) One Shot®	K200-01	20
	pBAD/TOPO® ThioFu-sion™ Expression System	One Shot® TOP10	K370-01	20
	pBAD102 Directional TOPO® Cloning Expres-sion Kit	One Shot® TOP10	K4102-01	20
	pBAD202 Directional TOPO® Cloning Expres-sion Kit	One Shot® TOP10	K4202-01	20
	pBAD TOPO® TA Expres-sion Kit	One Shot® TOP10	K4300-01	20
Expression in mammalian cells	pcDNA™3.3 TOPO® TA Expression Kit	One Shot® TOP10	K8300-01	20
	pcDNA™3.2/V5/GW/D-TOPO® Cloning Kit	One Shot® TOP10	K2440-20	20
	pcDNA™6.2/V5/GW/D-TOPO® Cloning Kit	One Shot® TOP10	K2460-20	20
	pcDNA™3.1 Directional TOPO® Expression Kit	One Shot® TOP10	K4900-01	20
	ViraPower™ HiPerform™ Lentiviral Expression Systems	One Shot® Stbl3™	K5310-00 K5320-00 K5330-00 K5340-00	1 kit
Entry into Gateway® expression systems	pCR®8/GW/TOPO® TA Cloning® Kit	One Shot® Mach1™-T1 ^R	K2520-20	20
		One Shot® Mach1™-T1 ^R + PureLink™ Quickprep Plasmid Miniprep Kit	K2520-02	20 rxns + preps
		One Shot® TOP10	K2500-20	20
	pENTR™/TEV/D-TOPO® Cloning Kit	One Shot® Mach1™-T1 ^R	K2535-20	20
		One Shot® TOP10	K2525-20	20
	pENTR™/D-TOPO® Cloning Kit	One Shot® Mach1™-T1 ^R	K2435-20	20
		One Shot® TOP10	K2400-20	20
	pENTR™/SD/D-TOPO® Cloning Kit	One Shot® Mach1™-T1 ^R	K2635-20	20
		One Shot® TOP10	K2420-20	20

For all of our Custom Services options, visit www.invitrogen.com/customservices.

References

1. Bernard, P. and Couturier, M. (1992) *J Mol Biol* 226: 735-745.
2. Bernard, P. et al. (1993) *J Mol Biol* 234: 534-541.
3. Rand, K.N. (1996) Elsevier Trends Technical Tips Online.